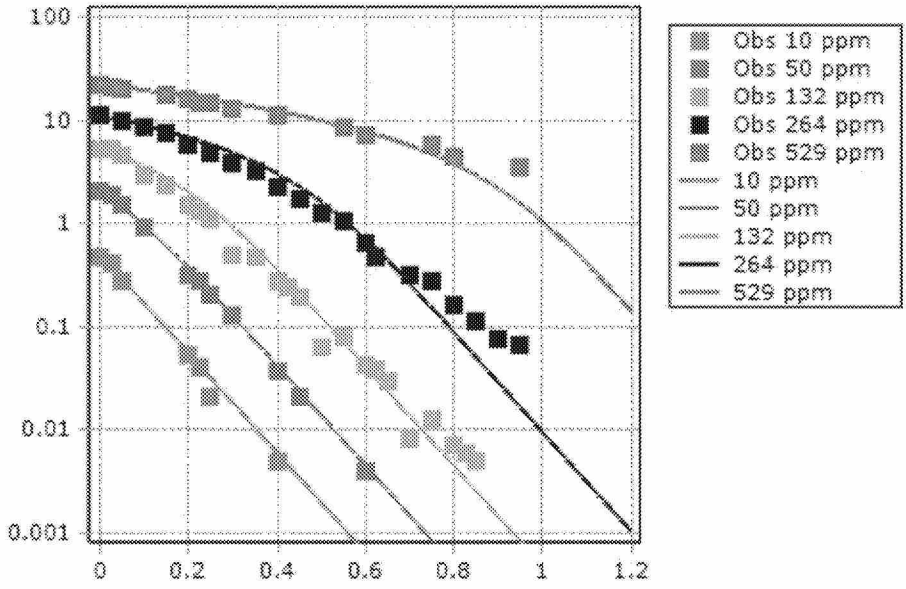

Based on this, I'd say that there's a very good chance that the gas-liquid mass transfer is a significant factor, and is likely to impact the estimation of Km (perhaps the goodness of fit of the fixed-Km model). The difficulty is that we need control incubation data to determine the correct value of KGL.

-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Tuesday, August 28, 2018 11:07 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Yes, it should be +ARLOSS. I must have hit the wrong key yesterday when I noticed it was missing from the equation.

Jerry Campbell
Managing Consultant

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jcampbell@ramboll.com

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, August 28, 2018 8:42 AM
To: Jerry Campbell <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Thanks, Jerry. I've forwarded to Alan who is getting back to his evaluation of the primary model. I'm hoping we can get through the model code evaluation by the end of next week…

Well, I just looked at the .csl and see this:

```
!MASS BALANCE
  CHECK1 = A10 - (A1+A1M+A1I+ ARLUNGVK-ARLOSS)
```

But that should be +ARLOSS?

-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Monday, August 27, 2018 4:30 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Harvey Clewell <HClewell@ramboll.com>
Subject: Chloroprene In Vitro model

Paul,

I've uploaded a zip folder (INVITROMODEL AND GRAPHS.zip) with the full workspace for the in vitro model and Excel files with the figures. There is a spreadsheet with a list of the m-files and a short description. Let us know if something doesn't work or you have any questions.

Jerry Campbell

Managing Consultant

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```
PROGRAM: INVITRO.CSL
!MODIFIED FROM PAUL INVITRO MODEL FOR MALE
!MODEL TO SIMULATE BETA CHLOROPRENE UPTAKE
!AND METABOLISM IN A ONE-COMPARTMENT
!REMOVE ALL THE PATHWAY RELATE TO CEO
!COSIDER SATURABLE PATHWAY FOR MICE LIVER/LUNG, RAT LIVER
!BUT ONLY V/K FOR RAT LUNG
!VIAL SYSTEM CONTAINING MICROSOMES ONLY
!VK RXN only happen in Rat Lung
!Including Background Rate : 0.001424477


VARIABLE TIME

INITIAL
dimension ts(30)
integer ns, ni
CONSTANT VMAX1=0.          !MAX RATE OF MET. (uMOL/HR/mg protein)
CONSTANT KM1=0.1           !MICHAELIS CONSTANT (uMOL/L)
CONSTANT RLOSS = 0.001424   !Background loss rate (1/hr)
CONSTANT VK=0.              !REPRESENT THE V/K COEFFICIENT FOR RAT LUNG (1/hr)

CONSTANT P1=0.69           !MEDIA/AIR PARTITION for CD

CONSTANT A10=0.            !INITIAL AMOUNT IN VIAL (uMOL)

CONSTANT VVIAL=0.01165     !VOLUME OF VIAL (L); Vial volume= 11.65 ml
CONSTANT VMED=0.001        !VOLUME OF MEDIA (L); Liquid voume
 VAIR=VVIAL-VMED     !HEADSPACE
CONSTANT PROT = 1.0        !AMOUNT OF PROTEIN (mg)

CONSTANT TF=0              !TIME OF FIRST SAMPLE (hr); kept same
CONSTANT TI=0.2            !INTERVAL BETWEEN SAMPLES (hr)kept same
CONSTANT VINJ=0.0002       !INJECTION VOLUME (L); based on Matt email
constant kgl = 0.026! gas-liquid transfer rate (L/h): from Schlosser et al. (1993)
constant set10 = 0.0! Initial air conc (uM) when not distributed to liquid first
constant TS = 30*0.0 ! sampling times (h)
constant NS = 6 ! # of sampling times in TS

!Initial Conditions
 CA10=A10/(VAIR+P1*VMED)
ag10 = (ca10+set10)*vair! PS, 8-28-2018
 CM10=CA10*P1     !CONC in SOLUTION
am10 = cm10*vmed! PS, 8-28-2018
! CA1=CA10
! CM1=CM10

A1I = 0.0! Initial amount taken by sampling
```

```
!TIMING COMMANDS
CONSTANT TSTOP=1          !LENGTH OF EXPOSURE (HOURS)
CONSTANT POINTS=100.      !NO. OF POINTS IN PLOT
CINTERVAL CINT=0.01       !COMMUNICATION INTERVAL

SCHEDULE step .AT. TS(1)
NI = 1
END                        !END INITIAL

DYNAMIC

ALGORITHM IALG=2

  DERIVATIVE

  TERMT(TIME.GE.TSTOP)

!CD KINETICS (umoles/hr)
  rgl = kgl*(ca1 - cm1/p1)! PS, 8-28-2018
agl = integ(rgl,0.0)
  R1M = VMAX1*CM1*PROT/(ABS(KM1+CM1)+1.e-16)
A1M = INTEG(R1M,0.0)
  RRLUNGVK=VK*CM1
  ARLUNGVK=INTEG(RRLUNGVK,0.)
  RRLoss = RLOSS*Ca1*p1! made function of Ca1, PS, 8-28-2018
  ARLOSS = INTEG(RRLoss,0.)
rmed = rgl - r1m - rrlungvk! PS, 8-28-2018
  amed1=INTEG(rmed,am10)! PS, 8-28-2018
cm1 = amed1/vmed! PS, 8-28-2018
rgas = - rgl - rrloss! PS, 8-28-2018
agas1=ag10-agl-arloss-a1i! PS, 8-28-2018
ca1= agas1/vair! PS, 8-28-2018


  A1=amed1+agas1


!MASS BALANCE
  CHECK1 = A10 - (A1+A1I+A1M+ ARLUNGVK+ARLOSS)

DISCRETE step
PROCEDURAL
!Routine for sample loss
ainj = CA1*VINJ
    a1i=a1i+ainj
ni=ni+1
```

```
if(NI .LE. NS) THEN
    SCHEDULE step .AT. TS(NI)
end if
END                          !END PROCEDURAL
END                          !END DISCRETE

END                          !END DERIVATIVE
END                          !END DYNAMIC
END                          !END PROGRAM
```

```
prepare @all
Male2008


 VVIAL=.01165;
 VMED=.001;
 VINJ=0.0002;
 VAIR=VVIAL-VMED;
 TSTOP=1.2; CINT=0.02; MAXT=0.001;
 PROT = 1.0;
 P1=0.69;
%Male Mouse Liver
dat=B6MmiceLiver; nexp=size(dat,2);
KGL=0.26

for pp = 2:nexp
       A10 = dat(1, pp)*(VAIR+P1*VMED);
     VMAX1=0.26; VK = 0.0; KM1=1.36;
KM1=0.8;
TS(1:length(TS))=TSTOP+(1:length(TS));
ts = dat(dat(:,pp)>0,1); NS=length(ts);
ts = ts(2:NS); NS=NS-1, TS(1:NS)=ts;

   start @nocallback
%plot(_time,_a1i*100)
    if pp==2
t1=_time; c1=_ca1;
elseif pp==3
t2=_time; c2=_ca1;
elseif pp==4
t3=_time; c3=_ca1;
elseif pp==5
t4=_time; c4=_ca1;
else
t5=_time; c5=_ca1;
end
end % end of dose loop
disp("Male mouse liver")
%save fkk @format=ascii @file=MM_liver_tab3.csv @separator=comma

%plot(dat(:,1),dat(:,2:nexp),t1,c1,t2,c2,t3,c3,t4,c4,t5,c5,'b6liver.aps')

for pp = 2:nexp
       A10 = dat(1, pp)*(VAIR+P1*VMED);
     VMAX1=0.21; VK = 0.0; KM1=0.95;
KM1=0.6;
TS(1:length(TS))=TSTOP+(1:length(TS));
ts = dat(dat(:,pp)>0,1); NS=length(ts);
```

```
ts = ts(2:NS); NS=NS-1, TS(1:NS)=ts;

   start @nocallback
%plot(_time,_a1i*100)
    if pp==2
t1a=_time; c1a=_ca1;
elseif pp==3
t2a=_time; c2a=_ca1;
elseif pp==4
t3a=_time; c3a=_ca1;
elseif pp==5
t4a=_time; c4a=_ca1;
else
t5a=_time; c5a=_ca1;
end
end % end of dose loop
plot(dat(:,1),dat(:,2:nexp),t1,c1,t2,c2,t3,c3,t4,c4,t5,c5,...
t1a,c1a,t2a,c2a,t3a,c3a,t4a,c4a,t5a,c5a,'b6liver.aps')
disp("Male Mice liver")
%save fkk @format=ascii @file=MM_liver_tab3_recalc.csv @separator=comma
```

Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 1/29/2019 7:31:53 PM
To: Harvey Clewell [HClewell@ramboll.com]
CC: Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Jerry Campbell [JCampbell@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; cvanlandingham@ramboll.com [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usereda39e51]; Sonja Sax [SSax@ramboll.com]
Subject: RE: Chloroprene In Vitro model

Thanks, Harvey. I had an email from Tina that I saw yesterday informing us of the delay, what was up (copied a memo from Denka). Are such incubations too old-school to even have the equipment on hand these days?!

Anyway, since I'm now in catch-up mode on various projects, and full government appropriations are still under negotiation, your expected timing is really not too bad for us. Still, I'll be very curious to see how it turns out when you get the results.

Best regards,
-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, January 29, 2019 1:52 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Sonja Sax <SSax@ramboll.com>
Subject: RE: Chloroprene In Vitro model

Hi Paul, glad you're gainfully employed again.

Now that you're back I wanted to update you on the status of the kg study. Jerry and I made changes to the experimental protocol to address your comments (modified protocol attached), and the laboratory determined that they could perform the revised protocol, but they also discovered that they needed to purchase a shaking water bath. Once that arrives, it will take them at least 2 weeks to conduct the lab work. I'll send you their report as soon as it's complete so we can discuss the results together. Then Jerry can re-estimate the metabolism parameters and incorporate them in the model. Although it is difficult to be precise with the schedule, we are optimistic about completing the PBPK model development in the next several months.

With kind regards
Harvey Clewell
PhD, DABT, FATS
Principal Consultant

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Research Triangle Park, NC 27709 USA
hclewell@ramboll.com

From: Harvey Clewell
Sent: Thursday, December 13, 2018 4:49 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <jcampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Sonja Sax <ssax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

Hi Paul

Here is the protocol from the laboratory that will be conducting the kg study. Please let me know if you have any comments or suggestions as soon as possible so we can get the study started. Feel free to call me if you want to discuss this.

On another note, have you ever heard back from Matt about finding the original reports on the metabolism studies he performed for his 2004 papers? As I recall, you're concern was to resolve the question of the sample volume for the male lung and liver studies. Were there any other concerns that the reports are needed for?

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Principal Consultant

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hclewell@ramboll.com

Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 7/16/2019 4:36:03 PM
To: Jerry Campbell [JCampbell@ramboll.com]
Subject: RE: model files

I thought so too, and it may be that DropBox just did this anyway.

Based on previous emails (from Harvey), the MCMC is just being done as a robust method of identifying a best parameter fit. These are not statistically meaningful given that the repeated measurement issue may not have been addressed; i.e., for the lung data, each concentration is only an 'n' of 1, not the number of time-points. So I don't consider the parameter distributions to be meaningful and wasn't planning to deal with them at all.

We would also have to look into rigorous measures of convergence also, beyond what's described in the papers, if we were to go there.

-Paul

From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Tuesday, July 16, 2019 11:09 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Subject: RE: model files

Sorry about that. I thought It would only count against my data. I had included the output files from the in vitro MCMC so you could review the posterior parameter distributions. You can delete them and just keep the pdfs of the posterior plots. It would save a lot of space.

Jerry Campbell

Managing Consultant

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Tuesday, July 16, 2019 10:49 AM
To: Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: model files

Jerry,

I was able to add it… you may have pushed me into a dropbox upgrade. :-/ But anyway I've pulled the zip file. Now I'll have to make time to get back into this!

-Paul

From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Tuesday, July 16, 2019 10:36 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Subject: model files

Paul,

I sent a share link to the model folder through dropbox. I did use your epa.gov email. I just noticed your dropbox account was a gmail address. I can switch addresses if you have trouble accessing the folder through your EPA address.

Jerry Campbell

Managing Consultant

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Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 8/15/2018 5:29:38 PM
To: Harvey Clewell [HClewell@ramboll.com]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]
CC: Robinan Gentry [rgentry@ramboll.com]; Allison Franzen [AFranzen@ramboll.com]; Miyoung Yoon [myoon@toxstrategies.com]; Sonja Sax [SSax@ramboll.com]; cvanlandingham@ramboll.com [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usereda39e51]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Jerry Campbell [JCampbell@ramboll.com]; Schlosser, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=121cf759d94e4f08afde0ceb646e711b-Schlosser, Paul]
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

I appreciate the reminder of these various factors. The critical question here is to what extent they apply to humans and can be predicted for us.

The "solubility", as quantified by the blood-air partition coefficient (PB), is low enough for CP (< 10) that "wash-in/wash-out" is unlikely to be a significant factor. However, if it was an explanation for reduced uptake in animals, then the factor would be assumed to also apply to humans, since it's determined by partitioning of the gas, not the specific exposure conditions.

And we have ruled out irritancy as an option. Anesthesia is also highly unlikely here.

Given that animals in a closed chamber may reduce respiration, that is a possibility. But given that irritancy and anesthesia are ruled out, this would be an effect of the 'housing', not the gas, hence the same degree of depression should occur for all exposure groups. If the adjustment is very different for different exposure levels, then the hypothesis of a chamber-specific effect would be ruled out. And as discussed yesterday, if V/Q ~ 1, there should be a concordant decrease in cardiac output. This adjustment would not be applied when analyzing internal doses for bioassay conditions and human exposures.

All that being said, we are first discussing part (1) of the QA: can we reproduce the Himmelstein et al. (2004) results using the Himmelstein et al. parameters. If we can't do that, then we might not actually have the correct parameters for that version of the model! How do we know there wasn't a mistake when listing them in the paper?

But then we'd also want to know, if the Yang parameters are to be considered, whether those gas uptake data can be matched using the Yang parameter set and (if necessary) a reasonable reduction in ventilation and cardiac output. If we can fit those data with the Himmelstein parameters but not the Yang parameters (short of unreasonable changes in ventilation, etc), then that will guide the choice of parameter sets. Likewise for consideration of the 'fixed Km' parameters.

-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Wednesday, August 15, 2018 12:13 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; cvanlandingham@ramboll.com; Davis, Allen <Davis.Allen@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Hi Paul

Hi Paul

One caveat when trying to apply the same PBPK model parameters across all studies is that ventilation/perfusion has been shown to be altered by the conditions of the animal exposure. This is of particular importance for interpreting the closed chamber studies conducted by Matt Himmelstein. As you have noted, Matt needed to decrease the values of QPC and QCC to fit his closed chamber data. This has been reported before for closed chamber studies. The following quotes are from Brown et al.(1997):

p.448:
Anesthesia has a profound effect on tissue perfusion, particularly that of cardiac and skeletal muscle and splanchnic and hepatic tissues. As a result, default values for these parameters in PBPK models of compounds that are not expected to exert an anesthetic effect should be based only on studies that used unanesthetized animals. Conversely, PBPK models of clinical anesthetic agents or environmental pollutants that exert an anesthetic effect should account for the potential effect of the anesthetic agent on tissue blood flow.

p.463 (note QA is QPC):
Despite the potential effect of inhaled compounds on respiratory dynamics, many PBPK models of volatile compounds use default values for QA, with little consideration of the potential error this may introduce into model-derived simulations. Johanson and Filser (1992) have demonstrated that use of the default values for QA proposed by Arms and Travis (1988) for mice and rats results in an overestimate of the clearance uptake measured using the closed-chamber gas uptake method. They found that experimentally determined clearance uptake values were about 60% of the values determined when the default reference values were used. Successful estimation of the experimentally derived clearance uptake values generally required the use of QA from about 30-80 mL/min for rats and about 5-14 mL/min for mice. Johanson and Filser (1992) offer three reasons for the necessity to use QA values that are about 60% lower than those proposed by Arms and Travis (1988) to successfully estimate experimentally derived clearance uptake values for animals in gas uptake studies. First, they point out that volatile compounds may act as respiratory irritants, which, as mentioned above, can reduce respiratory rate. Second, a wash-in/wash-out effect could be occurring where the volatile compound is absorbed onto the respiratory airways during inhalation and then desorbed during exhalation, resulting in reduced pulmonary uptake. Although alveolar ventilation may not be affected by the compound per se, a lower value for QA would enable clearance uptake from the chamber to be modeled more accurately. A third possibility, but one that Johanson and Filser (1992) consider remote, is the potential for volatile compounds to exert an anesthetic effect on the animals in the closed chamber, thereby affecting respiratory dynamics.
Regardless of the reason (direct effect on respiration or correction for a wash-in/wash-out effect), use of QA values that are 60% lower than those proposed by Arms and Travis (1988) may be necessary to accurately estimate clearance uptake of a number of volatile compounds in closedchamber gas uptake studies. These lower QA values have been used successfully by Johanson and Filser (1993) and Csanady et al. (1994) in PBPK models of butadiene and styrene, respectively. It is interesting to note that the alveolar ventilation rates derived by Medinsky et al. (1994) from a best-fit to the data in a PBPK model for butadiene are 17.1 and 70.8 mL/min for a 25-g mouse and a 250-g rat, respectively. These values are about 68 and 61 % lower than the reference values for this parameter suggested by Arms and Travis (1988). Nevertheless, many investigators have had success fitting closed-chamber data using alveolar ventilation values similar to those proposed by Arms and Travis (1988). For example, based on their experience, Clewell (1994) and colleagues found that the &dquo;best&dquo; values for alveolar ventilation were around 115 mL/min/100 g for the mouse and 35-50 mL/min/100 g for the rat.
Because PBPK models of inhaled compounds with high blood solubility are sensitive to values of QA, and because these same compounds have the potential to alter respiratory dynamics, it is

prudent to measure values for this parameter during exposures of animals or humans to these compounds (or at least to be aware of the effect that volatile compounds can have on respiration and to adjust the parameter values accordingly). Use of default values for QA in PBPK models of some inhaled compounds may result in overestimates of inhaled dose.

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, August 14, 2018 4:14 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

The QA process essentially has two steps:

1) Determine if we can replicate the original study, using those parameters "as is". What's been provided appears successful in this, though I don't see plots for the in vivo gas uptake data of Himmelstein.
2) QA the model code, parameters, data. In this case there is particular attention on the IVIVE. Is it truly predictive?

A component of (2) is tracing all model parameters back to their original source: the paper where the data was first collected/reported, or to a comprehensive physiological review. We have found this is particularly pesky for allometric coefficients, but other derived quantities can also be very hard to replicate. This is why we suggest (and in our own QA do) embedding calculations in a spreadsheet. The first column(s) are numbers exactly as you find them in the source, then the calculations. You've done this for metabolic constants, but not QPC/QCC.

If a parameter is just a little and the results is that there is only a modest fit in the plot of model simulations vs. data (eg, the Himmelstein gas uptake data are fit almost equally well with corrected parameters), that's fine. We then move ahead with the corrected parameters. The assumption is that had the revised plot been submitted for publication, it would have been accepted.

So for now, we aren't looking to refit parameters. But it would help to have the QPC/QCC solidly connected, calculations checked.

-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Tuesday, August 14, 2018 3:49 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; cvanlandingham@ramboll.com; Davis, Allen

<Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Hi Paul

I completely understand your concern, having had to review a number of poorly documented PBPK model manuscripts over the years. In the case of the chloroprene model, we were initially just trying to make sure we were correctly reproducing the models used in Himmelstein et al. (2004b) and Yang et al. (2012). Yang et al. provided new in vitro data and the PBPK model was used to illustrate of the predicted species differences in metabolism, but it was not intended to be a risk assessment paper. At this point we are ready to apply the model in a risk assessment and rather than just picking our own preferences we would like to discuss any suggestions you may have regarding changes to parameters where you believe there are better sources.

My understanding is that Allison corrected all the tissue weights and blood flows in our model to agree with Brown et al before we sent it to you, and I'm happy to continue to discuss the best approach for selecting and documenting the values for QPC and QCC. I'll ask Allison to look into your partition coefficient question.

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, August 14, 2018 3:00 PM
To: Harvey Clewell <HClewell@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

As far as I can tell, Matt H selected cardiac and ventilation parameters to fit his in vivo data for the mouse. Yang et al. (2012) lists the same parameters, did not appear to reconsider whether they are appropriate. The 'documentation' spreadsheet lists the same parameters and cites Himmelstein… who it seems incorrectly cites Brown et al. This is how errors can propagate, which is what we are trying to avoid and address via the QAPP. We have found somewhat frequently that a value in a PBPK paper was incorrectly transcribed from the original source, and sometimes it really does matter.

We are being especially careful here because this is the first time that a human PBPK model might be used without any human in vivo PK data for validation. The process and underlying calculations have to be rock solid.

In fact, if I go to the Andersen et al. (1987) methylene chloride paper (thanks for sending that), it does not list scaled QPC and QCC, but absolute rates, 2.32 L/h for the mouse. Given the BW of 0.0345 kg for the mouse in that paper, I get QPC = QCC = 2.32/(0.0345^0.75) = 29 (28.98 to be more exact).

The difference between 28, 29, and 30 is probably minimal. But for the purpose of the QAPP I need to trace the calculation from the actual source to the value being used, replicate the calculation. If it makes more sense to use the ventilation rates from the report in the docket, especially for simulating those data, we can go there, but then we'll go ahead and use the exact number (to 2 or 3 figures) we get from there.

We will need to consider what value is appropriate for simulating the bioassay conditions.

I will likely also check Astrand and Rodahl (1970). But, in your 2001 paper QPC = 24.0, QCC = 16.5, which does not match the values in the documentation/current model (27.75 and 12.89). And if I calculate 20 m^3 x 0.67/(24 * 70^0.75), the value I get for QPC is 23.1 (23.07), not 24.0.

Lastly, regarding the PC calculation spreadsheet, the table which lists the values for Himmelstein et al. (2004) has those numbers to like 14 decimal places; i.e., if one selects a value and looks at what's actually in the cell. I would guess that these are calculated values from the underlying original data. Do we have those data? If these are values as sent to you by Matt, that's OK, we'll just want to document that.

Thanks,
-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Tuesday, August 14, 2018 12:23 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; cvanlandingham@ramboll.com; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Hi Paul

I agree with your suggestion of changing the value of QCC in the mouse to 28 and citing Andersen et al. (1987). I'll add a discussion to the manuscript about the potential problems associated with using experimental measurements of cardiac output in the mouse for PBPK modeling of exposures. The methods used for estimating resting cardiac output in the studies cited in Brown et al were highly invasive, with a potential to disrupt normal physiology. This problem appears to be greater in mice than in rats, probably due in part to their small size. Regardless, the main point is that the measured resting values represent a basal perfusion rate that is not necessarily informative regarding experimental animals during an exposure. Mel Andersen came up with the idea of estimating liver blood flow (and thus cardiac output) by modeling data on chemicals under flow-limited metabolism conditions but we never published anything about it.

My comment about measuring ventilation was referring to the inhalation study we performed at the Hamner and submitted to the docket in 2010. I presented the results of our modeling of that study at the meeting last month. No parameters were fitted to the data from that study. We used the measured ventilation and assumed a V/Q of 1. The study you quoted in your email below was performed by Matt Himmelstein and published in 2004. I agree with you that the closed chamber data collected by Matt Himmelstein did not provide an adequate validation of the model because he was not able to measure the animals' ventilation rates. That was the impetus for measuring ventilation in the Hamner inhalation study.

Regarding the human, as you increase activity/workload the ventilation rate rises faster that cardiac output so V/Q becomes greater than 1. Fortunately, there is excellent data available from Astrand and Rodahl (1970) on both ventilation and perfusion as a function of workload. When I was running the PBPK model for vinyl chloride for the EPA IRIS assessment I went to the trouble of estimating ventilation and perfusion values at the two standard activity levels used in the risk assessment: EPA default (20 cu.m./day) and OSHA default (10 cu.m./8hrs):

```
PROCED EPA
SET QPC=24,QCC=16.5
END

PROCED OSHA
SET QPC=35,QCC=18
END
```

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To: Harvey Clewell <HClewell@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Schlosser, Paul <Schlosser.Paul@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

First, if the actual source of this value is other than Brown et al. (1997), then the actual source/citation should be provided.

But this is a direct quote from Himmelstein et al. (p. 30): "The physiological and metabolic parameters obtained from in vitro experimentation were not adjusted except for the alveolar ventilation (QPC) and cardiac output (QCC) as needed to adequately fit the experimental gas uptake data." Then on p. 32:

For both exposure systems, *in vitro* scaling of total CD metabolism was sufficient to explain the *in vivo* gas uptake data. The alveolar ventilation and cardiac output values used for simulation of the experimental gas uptake data were lower than the standard values used for dosimetry modeling (Table 1). The adjustment for the gas uptake simulations gave values for alveolar ventilation that were consistent with those used for modeling of various chemicals (Johanson and Filser, 1992; Medinsky *et al.*, 1994). Plausible explanations proposed by Johanson and Filser (1992) for using approximately 60% of the theoretical alveolar ventilation values reported by Arms and Travis (1988) included reduced ventilation due to sensory irritation, absorption and desorption by the upper airways, or anesthetic effects. For dosimetry modeling, the decision was made to assume the standard ventilation and cardiac parameters based on Brown *et al.* (1997) given the possibility that these parameters were more appropriate for estimating uptake and metabolism associated with bioassay conditions involving repeated whole body exposure.

This is roughly repeated in the discussion. So this contradicts your statements that ventilation was measured – there is nothing in the paper describing such measurements, and if it was there would be no reason to cite Johanson and Filser, Medinsky, since then he would have just used the value he measured. This says pretty clearly that these parameters were adjusted to fit the in vivo PK data (and then switched to more standard values for bioassay simulations).

If Andersen et al. (1987) provides supporting science for using a higher QCC, then that should be cited, so we can go to and check that reference. If there is a significant error in a primary source for physiological parameters (Brown et a.), then that should have been published at some point. While I know that you and Mel did a lot of this early work, we need peer review citations to meet the requirements of our QAPP.

We can potentially use the value of 28 from Andersen et al. (1987).

All that being said, if it is true that V/Q should be closer to 1, then a value of 2.15 for humans should not be used. I think it would be defensible to apply the same V/Q for humans as needed to fit the mouse data.

-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Monday, August 13, 2018 4:54 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; cvanlandingham@ramboll.com; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Hi Paul

The value of QCC for the mouse in the chloroprene model (QCC=30), is similar to the mouse value (QCC=28) in the PBPK model of Andersen et al. (1987) that was used by EPA in the IRIS assessment for methylene chloride, and is consistent with the physiology of ventilation and perfusion.

I was a member of the ILSI RSI committee that resulted in the publication of Brown et al. (1997), and the question of the correct value of QCC to use in a PBPK model for the mouse was a point of discussion at that time. As mentioned in the section beginning on p.453 of Brown et al., while the value of cardiac output used in the PBPK model of Andersen et al. (1987) for the rat is in agreement with the experimental measurements reported in Table 22, the value for the mouse is about double the reported values. The decision to use the higher value of QCC in the mouse was made by Mel Andersen and I when we were at Wright-Patterson AFB, and was the result of comparisons of PBPK models with data for a number of chemicals.

If you convert the alveolar ventilation rates in Table 31 to the same units as the cardiac output in Table 22 (mL/min), the experimental value of 14 mL/min for a 23-30g mouse that is reported in Table 22 of Brown et al. is inconsistent with the experimental value for the ventilation rate in the mouse in Table 31, and would result in a mismatch between ventilation and perfusion (V/Q ratio). Apart from situations involving strenuous activity or disease, ventilation and perfusion rates are maintained at a V/Q ratio close to 1, and a departure from this value by more than 20% is considered of clinical significance. While the data from rats and dogs are consistent with a V/Q ratio close to unity, the mouse data are not.

Species	Alveolar Ventilation (mL/min/100g) (Table 31)	BW (g)	Alveolar Ventilation (mL/min)	Cardiac Output mL/min (table 22)	V/Q ratio
Mouse	116.5	30	35	14	2.50
Rat	52.9	250	132	110	1.20
Dog	23.1	15000	3465	2936	1.18

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Sent: Monday, August 13, 2018 9:41 AM
To: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Jerry, Harvey,
Cc: Also including EPA colleagues, managers

The attached goes on to describe a couple of discrepancies/issues for the physiological parameters, for the most part minor. However, there is a major issue with the QCC for the mouse:

"… for the mouse the QCC and BW yield a total cardiac output of 36 ml/min, while Table 22 of Brown et al. (1997) gives a mean of 14 ml/min, with a range of 12-16 ml/min. Hence the QCC is unrealistically high, should be ~ 11.7 L/h/kg$^{0.75}$. But using QCC=11.7 in the female_mouse_invivo_3.R script results in significant over-prediction of the blood concentration data. This indicates a failure in in-vitro to in-vivo extrapolation, since the increase in QCC effectively increases the rate of metabolism (when flow-limited) to a similar extent. At a minimum, the "parallelogram" approach suggests that a similar correction, a factor of 2.6 times the mean, should be applied for the human QCC when calculating human internal doses."

It's possible that there's a mistake in the in-vitro to in-vivo metabolic extrapolation/calculations that you all can correct. But I flicked through that part of the 'documentation' spreadsheet and see that the calcs are embedded, so I expect all of those check out. What's written above re. a parallelogram option is just my take for possibly dealing with the discrepancy, but we'd need to have an internal discussion about that before determining if it's acceptable.

Also, please provide the full citation for "Clewell et al. (2001)", listed for human physiological parameters. And as indicated before, the spreadsheet refers to another sheet for calculation of the partition coefficients, which wasn't included.

Best regards,
-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Monday, August 06, 2018 9:30 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; cvanlandingham@ramboll.com; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

I was just getting to that option. See if this will work.

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Managing Consultant

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Monday, August 06, 2018 9:26 AM
To: Cynthia Van Landingham <cvanlandingham@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Try just changing the file-extension from .zip to .txt and sending as an attachment. I'm trying to unzip the thing from the sharepoint site and just getting a spinning wheel.

From: Cynthia Van Landingham [mailto:cvanlandingham@ramboll.com]
Sent: Monday, August 06, 2018 9:19 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Unfortunately, I believe that the restrictions are on your end not ours. We can all see the files no problem.

Cynthia

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Monday, August 06, 2018 8:18 AM
To: Cynthia Van Landingham <cvanlandingham@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

I tried to just download it. Does it have to be this complicated? We'll be sharing with everyone as part of our open and transparent process…

-Paul

From: Cynthia Van Landingham [mailto:cvanlandingham@ramboll.com]
Sent: Monday, August 06, 2018 9:13 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Paul,
Did you download the zip file to your hard drive and then open or did you open it on the OneDrive site? If you did not try this, selecting all the files and allowing OneDrive to produce one download zip may be best. The

chloroprene_model.o_error.txt file is not in the zip we created so may be something that is being created due to the download process. Please read that file to find out if your IT security set-up is preventing files from being extracted.

Thanks, Cynthia

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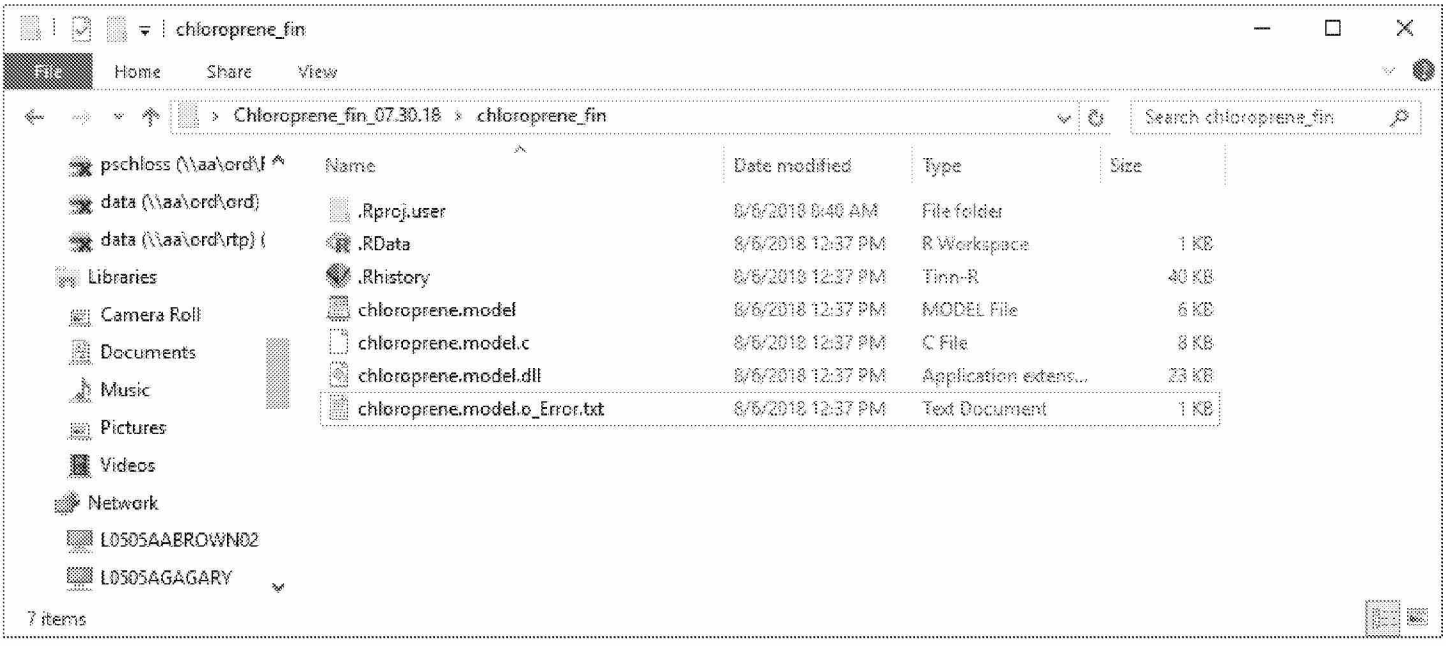
From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Monday, August 06, 2018 7:53 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

I sent a separate email to Alison. Below is a screenshot of the model folder that I got. There are none of the scripts listed in the Excel 'documentation' file.

Once we have those, give us some time to look at it. Hopefully it's easy enough to figure out, but we can let you and Jerry know if we need a walk-through.

-Paul



From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Friday, August 03, 2018 2:02 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Allison Franzen

<AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: transmission of PBPK model for chloroprene

Hi Paul

As promised, we are providing you with the PBPK model for chloroprene written in R, with all the associated scripts and documentation. You should have received a separate email with an invitation to access the files on Microsoft OneDrive. Please let me if you have any problem downloading or opening them. Jerry Campbell would be happy to come over to EPA to help you set up the run environment in R studio and answer any questions you may have about running the model.

I'm looking forward to talking with you about the model and discussing any questions, suggestions, or concerns regarding it. Would it be possible to arrange an initial meeting sometime in the next few weeks. Miyoung Yoon is completing her review of the metabolism parameter scaling approach and I would like to be able to include you in the discussion of her recommendations.

Harvey Clewell

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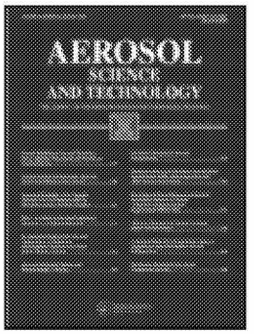

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Lung Dosimetry: Pulmonary Clearance of Inhaled Particles

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Lung Dosimetry: Pulmonary Clearance of Inhaled Particles

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In lung dosimetry of inhaled particulate compounds one has to consider both deposition and clearance kinetics throughout the respiratory tract. The deposition is governed essentially by particle size, ventilatory parameters, as well as airway characteristics, whereas the clearance of particulate compounds, once deposited, is dependent on physicochemical characteristics of the compound. Predictive models of particle deposition for specific regions in the lung or specific airway generations can be applied to estimate regional and local doses of an inhaled compound. The main clearance mechanism for insoluble particles deposited in the conducting airways is via the mucociliary escalator. Soluble particles are cleared mainly by diffusional and pinocytotic processes from this region, depending on their lipo- or hydrophilicity. The main clearance mechanism for insoluble particles in the alveolar region is based on the function of the alveolar macrophages that effectively phagocytize deposited particles and transport them toward the mucociliary escalator. Soluble compounds deposited in the alveolar region will mainly be cleared by diffusional and pinocytotic processes via inter- or transcellular pathways where lipophilicity, hydrophilicity, and molecular size play an important role. Knowledge of the retention characteristics of specific inhaled compounds is important for developing strategies for targeting regions of the respiratory tract. In addition to applications in diagnostic and therapeutic aerosol delivery, knowledge of lung dosimetry is also important for extrapolation of results from animal studies to humans, in particular when applied to toxicological investigations. Using inhaled particles as carriers for other chemical compounds or modulating uptake mechanisms should also be considered as a means for increasing the pulmonary retention of otherwise rapidly cleared compounds. Lung clearance and retention processes of inhaled particulate compounds may be significantly altered in the diseased lung, thereby changing the clearance kinetics of soluble and insoluble particulate compounds, which has to be considered in pharmaceutical applications.

INTRODUCTION

This brief review is intended to describe only some basic facts with respect to the clearance and retention of inhaled particles, insoluble and soluble, in the lower respiratory tract. Knowledge about such basic facts is important for an understanding of the dosimetry in the lung. Information of the retention characteristics of specific inhaled compounds is important for developing strategies for targeting regions of the respiratory tract both for the application of therapeutic and diagnostic aerosols. In addition to applications in diagnostic and therapeutic aerosol delivery, knowledge of lung dosimetry is also important for extrapolation of results from animal studies to humans, in particular when applied to toxicological investigations. One has to consider furthermore that basic clearance and retention processes of inhaled particulate compounds may be significantly altered in the diseased lung, thereby changing the clearance kinetics of both soluble and insoluble particulate compounds.

REVIEW AND DISCUSSION

Figure 1 shows a general scheme about exposure dose–effect relationships of inhaled substances as it relates to res-

Aerosol Science and Technology 18:279–289 (1993)

0278-6826/93/$6.00

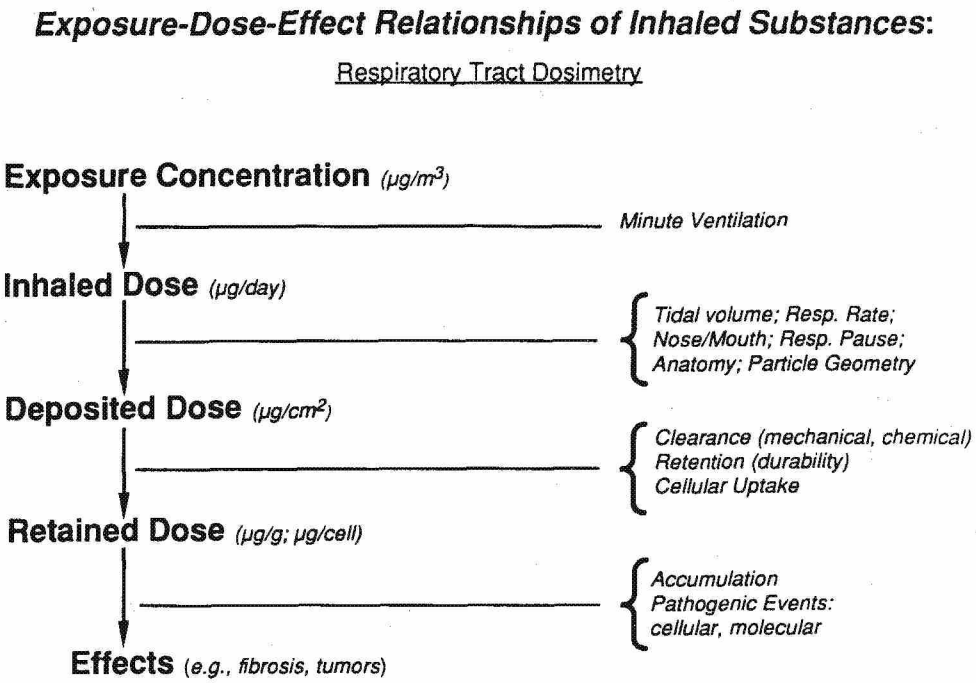

FIGURE 1. Exposure dose–response relationships applicable for dosimetry of the respiratory tract.

piratory tract dosimetry. The exposure is determined by a certain exposure concentration which, depending on the minute ventilation, leads to a certain inhaled dose that can be expressed in micrograms inhaled per day. Depending on parameters such as tidal volume, respiratory rate, nose or mouth breathing, a respiratory pause, the anatomy of the airways, and geometry of particles, a fraction of the inhaled dose will be deposited in the respiratory tract. Once deposited, specific clearance mechanisms will become effective, leading to a clearance of the deposited substances or a retention in different compartments of the respiratory tract. This retention then results in a retained dose in these areas in the lung which may eventually, after a certain period of accumulation and based on cellular and molecular mechanisms, lead to effects in the lung which could be either acute (inflammation) or more chronic (fibrosis, tumors). Thus, the major components of the dosimetry in the respiratory tract are (1) the deposition of inhaled compounds and (2) the retention of those compounds in specific lung regions. With respect to deposition, as indicated above, ventilatory parameters and particle characteristics become the major determinant. For larger particles ($> \sim 2$ μm) the nose resembles a very effective filter. Obviously, mouth breathing circumvents this filtrating capacity so that the lower respiratory tract will receive a greater dose with this mode of breathing. Deposition in the alveolar space is maximum for particles with an aerodynamic diameter of ~ 3 μm, whereas a minimum of deposition occurs for particles ~ 0.5 μm because the deposition mechanisms of sedimentation and diffusion are minimal for this particle

size (for summary on deposition see Morrow and Yu, 1985).

With respect to clearance mechanisms in the lower respiratory tract, Figure 2 shows a schematic overview for elimination of inhaled insoluble and soluble particles. Because virtually any type of particle is to at least a very minimal degree soluble in the lung, the term "insoluble particles" should refer to such particles whose solubilization rate is insignificant compared to the long-term mechanical clearance rate, e.g., TiO_2 particles. The main clearance mechanism in the conducting airways is the mucociliary escalator, where a constant current of mucus flow is created via ciliary beating. The cilia themselves are moving in a so-called sol layer and, depending on their beat frequency and stroke amplitude, move a

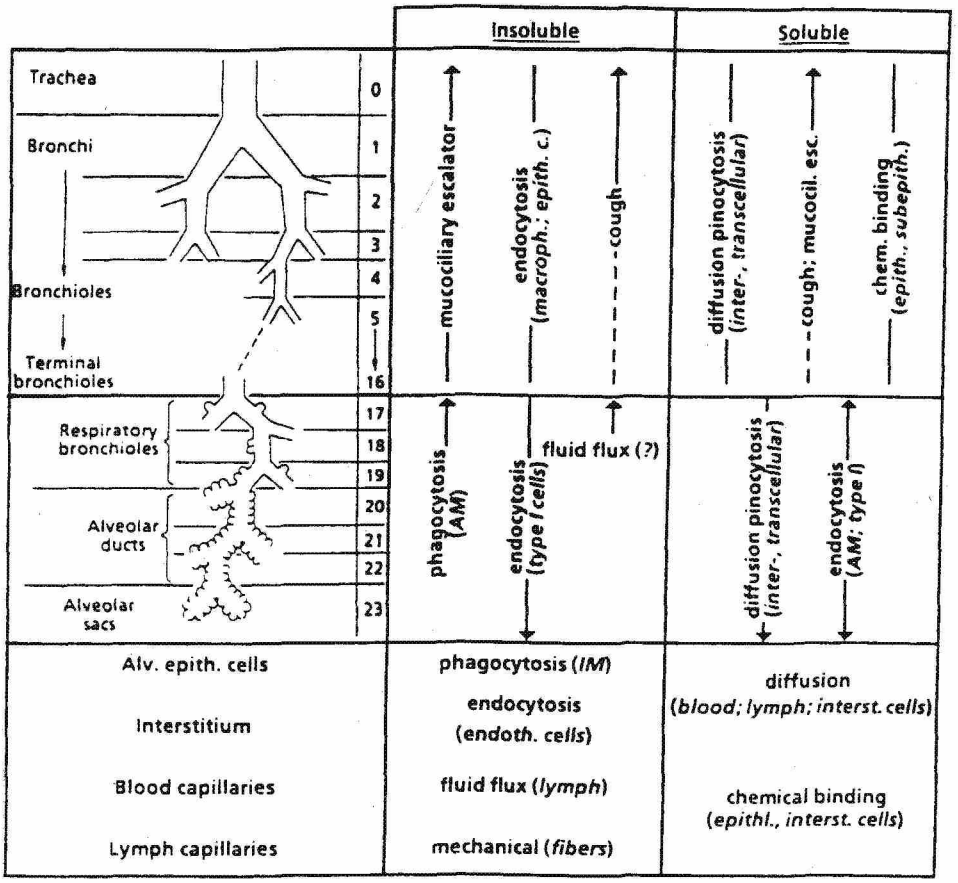

FIGURE 2. Schematic representation of major clearance mechanisms in the lower respiratory tract. The conducting airways (generations 0–16) and alveolar region (generations 17–23) and lung tissue are represented separately (from Oberdörster, 1988). Tissue components are listed in the last row, column 1, and respective clearance mechanisms in columns 2 and 3. The arrows indicate movement of particles into different compartments (conducting airways, alveolar airways, lung tissue).

gel layer on top towards the larynx. Particles depositing on this layer are moved with the flow of mucus. It is generally thought that mucociliary clearance is rather effective and completed within 24 h. However, newer evidence suggests that particles also may be displaced toward the epithelial cells via surface active forces stemming from a surfactant layer on top of the gel layer, as recently described by Gehr et al. (1990). Airway macrophages then may phagocytize those particles and lead to a long-term retention in this region. A longer-term retention phase for deposited particles has been discussed by Stahlhofen et al. (1990) based on measurements in human volunteers, and although underlying mechanisms are not completely understood, the engulfment of particles by airway macrophages and subsequent retention inside macrophages or also the displacement of particles into the sol layer might be contributing factors for such long-term particle retention in the conducting airways. Uptake into airway epithelial cells has also been described (Sorokin and Brain, 1974). Another rather effective mechanism for clearing particles out of the conducting airway zone is via coughing; however, this might be most effective only for the upper generations of airways. With respect to soluble particles depositing in the conducting airways, diffusion via intercellular pathways and pinocytotic processes leading to transcellular clearance may take place.

In the alveolar region of the lung, the most prominent clearance mechanism for insoluble particles is uptake by alveolar macrophages and subsequent clearance via movement of those cells towards the mucociliary escalator. This process may take a long time depending on the site of particle-alveolar macrophage encounter, and also possibly depending on the extent and length of respiratory bronchioles connecting the alveolar ducts with the terminal bronchioles where the mucociliary removal mechanism begins. For particles that are not phagocytized by alveolar macrophages, endocytosis by type I cells has been described. This will most likely occur, for example, in a situation of particle overloading of the alveolar region or in the case of fibers that cannot be fully phagocytized by alveolar macrophages. This epithelial uptake leads subsequently to the access of those endocytosized particles into the pulmonary interstitium, where in turn they can interact with interstitial cells, i.e., interstitial macrophages or fibroblasts. Soluble particles in the alveolar space are mainly cleared by diffusional and pinocytotic processes via inter- and transcellular pathways. In the interstitium, uptake into the blood and lymph system via diffusion may occur, and depending on the chemical composition of the material, chemical binding to lung cells may also occur, e.g., with epithelial or interstitial cells. The following paragraphs delineate some of these basic clearance mechanisms more closely.

Alveolar macrophage–mediated clearance of highly insoluble particles in the alveolar region of the lung is quite different among the different mammalian species. Figure 3 shows such clearance for

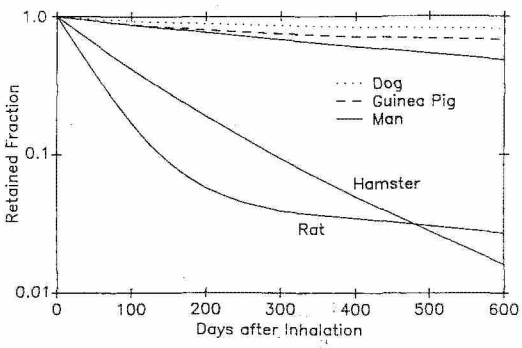

FIGURE 3. Species differences in alveolar macrophage–mediated clearance of highly insoluble particles as described by Snipes et al. (1983) and Bailey et al. (1985a, b).

several rodent species, dogs, and humans. Whereas hamster and rat clear particles very quickly from their lungs, the dog, guinea pig, and human clear those particles much more slowly. The reasons for this are unknown, and differences between those species in the extent of respiratory bronchioles alone cannot be responsible: Both hamsters and guinea pigs have about one generation of respiratory bronchioles yet their long-term clearance rates are quite different. (For more information on the extent of respiratory bronchioles, see Phalen and Oldham, 1983.) The mechanical clearance rate for these particles is not constant over time as can be seen most prominently for the rat in Figure 3. In fact, long-term particle retention studies in humans performed by Bailey et al. (1985b) with highly insoluble particles show that the clearance rate changes from about 4×10^{-3} per day immediately after exposure to about 1×10^{-3} per day by day 300 or 400 after inhalation. The corresponding retention halftime in humans for such particles changes from about 170 days shortly after exposure to about 700 days 1 year after the exposure. This change in clearance rate over time has now been incorporated into a clearance model proposed by a study group of NCRP (Cuddihy et al., 1988).

Alveolar-macrophage-mediated clearance of highly insoluble particles involves several steps. First, particles that have deposited in the alveolar region have to be recognized by alveolar macrophages. It has been suggested by Warheit et al. (1988) that the induction of locally generated chemotactic factors, possibly originating from the complement cascade involving C5a, facilitate particle–macrophage encounter. The actual process of phagocytosis consists of the attachment, which may be mediated by specific receptors, and the internalization process which may be dependent on the size of the particles. During internalization, a phagosome is formed which becomes a phagolysosome with the influx of lysosomal enzymes for digestion of particles. This process of phagocytosis leads to an activation of alveolar macrophages, resulting in an oxidative burst with release of mediators including oxygen radicals, chemotactic factors and growth factors which can interact with other cells in the lung (Holian and Scheule, 1990). Depending on the type of the phagocytized particle, macrophages will either effectively clear those particles toward the terminal bronchioles as indicated above, or they may also become severely damaged and die, e.g., when crystalline silica particles are phagocytized. It has also been suggested that macrophages carry their particle burden across the epithelium into the interstititum (Harmsen et al., 1985) from where these particles may eventually end up in the thoracic lymph nodes. However, this process will only be of minor importance involving only a very small fraction of particle-carrying alveolar macrophages and it does not constitute a major clearance mechanism.

Similar to the major differences in particle retention between species shown in Figure 3, significant differences in alveolar macrophage–mediated particle clearance have also been observed within the same species when different-sized particles were administered to the lungs. For example, instillation of radiolabeled polystyrene particles of diameters 3.3 or 10.3 μm into rats showed that only the smaller particles were cleared in the rat with the expected retention halftime of about 70 days, whereas the larger particles were hardly cleared at all (Figure 4) (Oberdörster et al., 1992b). This phenomenon of particle size–dependent clearance in the alveolar region is based on the concept of lung particle overload. In such overload conditions, excessive amounts of highly insoluble but otherwise

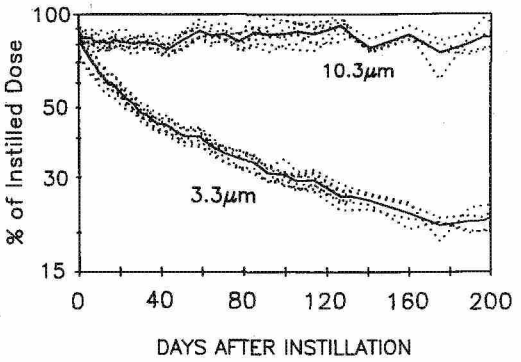

FIGURE 4. Pulmonary retention of large and small insoluble polystyrene particles in the rat (from Oberdörster et al., 1992b).

biologically rather inert particles have been found to lead to a cessation of alveolar macrophage-mediated particle clearance (Muhle et al., 1990). Morrow (1988) has proposed that this is due to a volumetric overload of alveolar macrophages, i.e., if the phagocytized volume of particles approaches ~60% of the normal alveolar macrophage volume those cells become immobile and will not clear effectively the particulate burdens. In that the normal alveolar rat macrophage volume is ~1000 μm^3, one particle of 10.3 μm in diameter represents a volume of ~600 μm^3, and if phagocytized by a macrophage should cause an overload-induced retardation of clearance according to Morrow's hypothesis. While the studies with small and large particles (Figure 4) seem to support this hypothesis, it has to be considered that phagocytosis of many small particles occurring under realistic conditions and leading to the same 600-μm^3 macrophage burden will be different in terms of macrophage fluidity, and also be different with respect to redistribution of such particles once the overloaded macrophages die. As pointed out by Lehnert (1990) redistribution of particles after the death of macrophages plays an important role in diminishing the particle burden of individual macrophages by reuptake of those particles by other macrophages. This may eventually lead to a restoration of clearance mechanisms, although studies by Bellmann et al. (1991) indicate that once a heavy overload situation in the lung has occurred clearance may be persistently retarded, even months after exposure to the overloading particulate material has ceased.

The dependence of the clearance and translocation kinetics of highly insoluble particles from and within the alveolar space on particle size becomes quite obvious for very small particles which are more relevant since they are respirable, in contrast to 10-μm particles. Recent studies in our laboratory with ultrafine particles (i.e., TiO_2 particles with diameter of ~20 nm) showed that these particles appear to be less effectively phagocytized by alveolar macrophages which led to an increased interstitial access of those particles via increased epithelial transfer. Subsequently, we could observe an increased uptake into lymphatic tissue as well and, most importantly, we could demonstrate that such ultrafine particles compared to larger-sized particles also lead to increased inflammatory reactions in the lung which could result in long-term adverse effects (Oberdörster et al., 1990, 1992a; Ferin et al., 1991, 1992). The increased pulmonary toxicity of the ultrafine particles seems to be related to the larger particle surface area. Underlying mechanisms—e.g., interaction with macrophage receptors—need to be investigated further.

As mentioned above, alveolar clearance of soluble particles occurs mainly via diffusional processes. Cellular uptake of such compounds depends on their molecular weight, hydrophilicity, and lipophilicity. However, chemical binding to specific cell structures and proteins may also occur, e.g., inhalation and pulmonary retention of cadmium compounds. With re-

spect to hydrophilic compounds, a strong correlation of their retention in the alveolar space with molecular size has been found (Jones et al., 1982). Figure 5 shows schematically such correlation, comparing solute transfer characteristics of the alveolar epithelium with the pulmonary capillary endothelium. Tight junctions between epithelial and endothelial cells differ significantly in that larger-sized molecular hydrophilic substances will only minimally penetrate the alveolar epithelium (reflection coefficient = 1) whereas such minimal penetration for the capillary endothelium occurs at much larger molecular sizes. For larger-sized molecules, e.g., proteins, transfer across the alveolar epithelium will mainly occur by pinocytotic processes. Damage of the alveolar epithelium caused by inhaled compounds or by excessive release of mediators from inflammatory cells, such as oxygen radicals or proteases, will result in a leaky epithelium allowing larger-sized solutes to penetrate this normally very tight barrier. Such leakiness has important implications for transfer of solute drugs in diseased states, in that solutes are then more efficiently

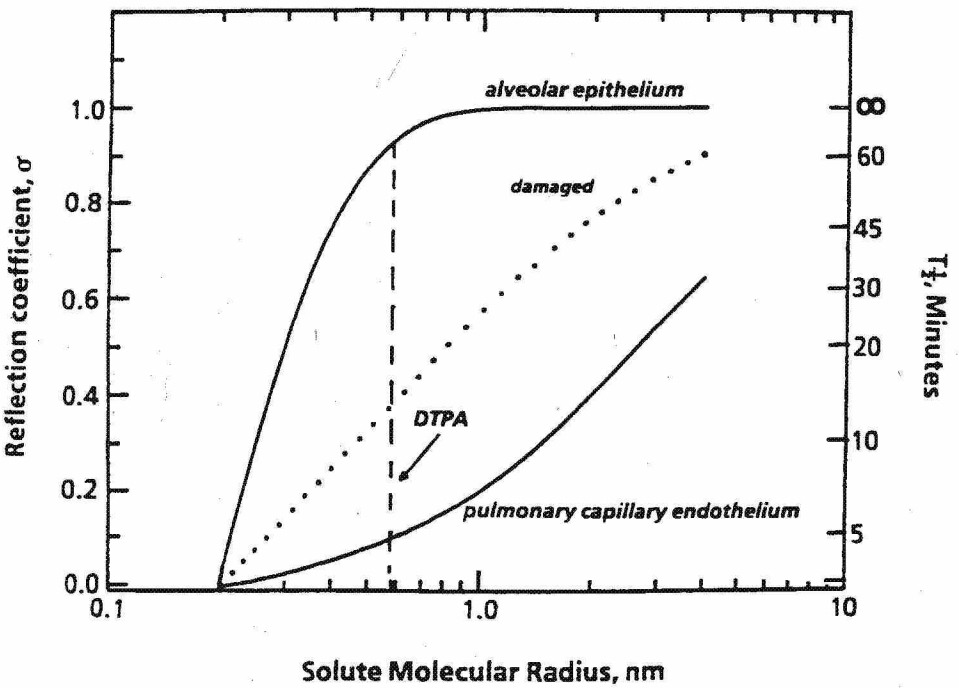

FIGURE 5. Hydrophilic solute permeability via tight junctions in alveolar epithelium and pulmonary capillary endothelium as a function of molecular solute radius (modified from Jones et al., 1982). The reflection coefficient σ is the ratio between the actual osmotic pressure and the ideal osmotic pressure. A value of 1 indicates no movement of the solute across the membrane. A tracer compound, DTPA, is cleared from the normal lung with a halftime of ~80 min, whereas damaged alveolar epithelium becomes much more permeable thereby decreasing the retention halftime, T 1/2 of DTPA.

eliminated from the lung compared to a healthy lung.

In contrast to hydrophilic solutes, lipophilic compounds are more easily taken up into cell membranes and cells. However, their clearance is highly dependent on their lipophilicity and molecular weight. In an experiment with a series of anthraquinone dyes, Henderson et al. (1988) found that increasing molecular weight and concomitant increasing lipophilicity was associated with longer retention in the lung (Figure 6). They found in these studies that the dyes with highest lipophilicity appeared to clear from the lung with long halftimes similar to water insoluble inorganic compounds. They suggested that the highly hydrophobic molecules will remain as lipid aggregates in the lung since they have a higher affinity for themselves than for the polar lipids in cell membranes. They concluded that on the basis of their results, organic soluble compounds with molecular weights < 300 daltons will clear the lungs rapidly, whereas nonpolar organic soluble compounds with a molecular weight > 300 daltons should clear the lungs more slowly.

In another series of studies investigating species differences in solute clearance, Schanker et al. (1986) found that a lipophilic compound is cleared in the lungs of mice, rats, and rabbits with the same rate, whereas a hydrophilic compound showed significant species differences, with the rabbit exhibiting a slower clearance than the rat, and the mouse showing the fastest clearance for this compound. On the other hand, water-soluble inorganic compounds may be cleared from the lungs much more slowly than one would predict based on their molecular weight alone. For example, metal salts like $CdCl_2$ which are highly water soluble are cleared from the lungs of rats, dogs, and monkeys with the same slow clearance rate as highly insoluble particles in these species (Oberdörster, 1988). This is due to the fact that these compounds can bind to proteins such as albumin or other inducible proteins such as metallothionein and are then stored in certain cell types of the respiratory tract from where they are slowly released.

As mentioned above, injured lungs show a very different clearance behavior compared to healthy lungs. Thus, if the lung is targeted for delivery of drugs by inhalation, when a disease such as an inflammatory process is present, the clearance

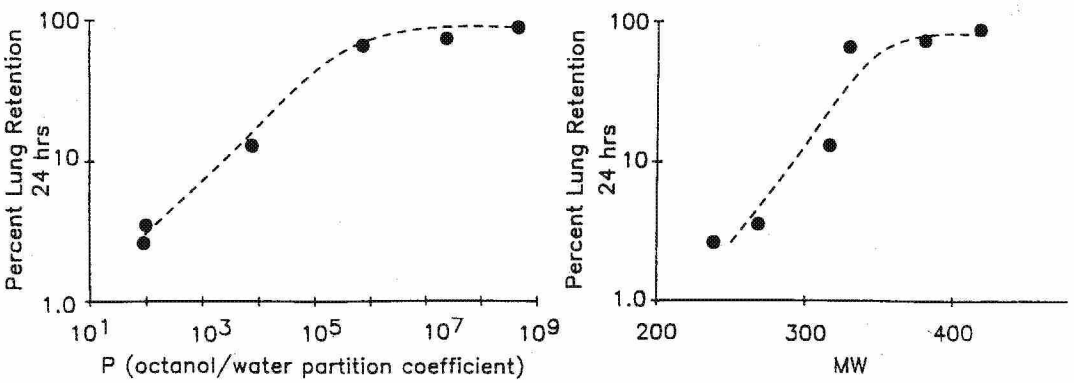

FIGURE 6. Lung retention of lipophilic compounds as a function of molecular weight and octanol/water partition coefficient. (Henderson et al., 1988, as determined with different anthraquinone dyes).

of the inhaled substance will change quite significantly compared to a healthy lung. Figure 7 compares the clearance of a solid particle and of a hydrophilic solute from the lungs of smokers and nonsmokers. Chronic cigarette smoking is known to induce inflammatory conditions in the lungs which will affect alveolar macrophage–mediated particle clearance and will also affect, either directly or indirectly via released mediators, the integrity and leakiness of the alveolar epithelial membrane. Consequently, solid particles will be cleared less effectively in smokers than in nonsmokers, whereas hydrophilic solutes deposited in the alveolar region will be cleared more rapidly in smokers' lungs as compared to nonsmokers (Figure 7). Such differences have to be considered when administering drugs to diseased lungs, either for therapy of lung injury or also if remote organs other than the lungs are targeted via the respiratory tract.

Knowledge of the pulmonary retention and clearance pathways of different types of inhaled solid and soluble compounds is necessary for targeting respiratory tract regions or modifying retention behavior. For example, retention of normally quickly cleared compounds from the lung can be increased by adsorbing such compounds to solid particles. Creasia et al. (1976) showed, for example, that benzo-a-pyrene (BaP) when administered to the lung was cleared very rapidly in mice. However, when BaP was co-administered together with carbon particles, retention of BaP was dramatically increased in the lung. A difference was even found whether the BaP was co-administered with small carbon particles (0.5–1 μm) or large carbon particles (15–30 μm) in which latter case the longest retention halftimes for BaP were observed in the respiratory tract (Figure 8). Retention of the large particles and concomitantly the adsorbed BaP may have been also influenced by the above-mentioned overload mechanism in the lung. However, while it is possible to modify retention characteristics of an inhaled compound, one has to evaluate carefully whether possible adverse reactions might have been introduced by such measures.

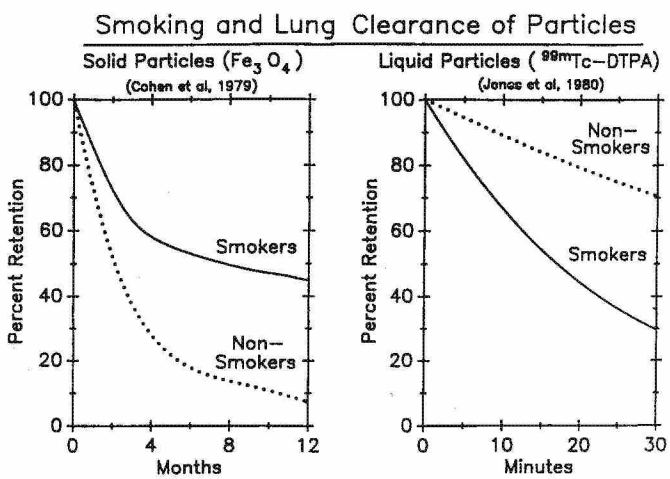

FIGURE 7. Lung clearance of solid and solute particles is significantly affected in the injured lung, here represented by smokers.

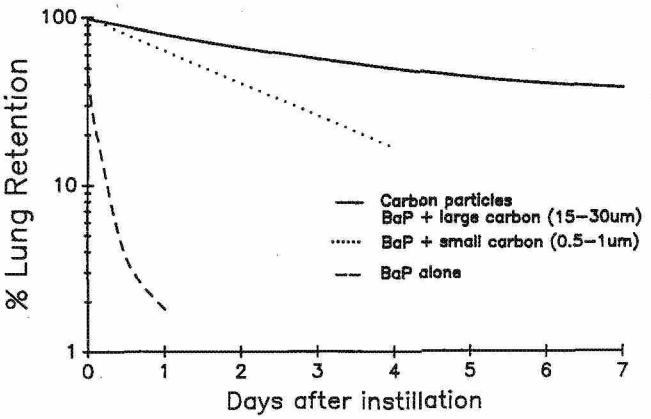

FIGURE 8. Significant prolongation of lung clearance of benzo-a-pyrene (BaP) after co-administration with carbon black particles (after data from Creasia et al., 1976).

Targeting specific lung compartments could be achieved by altering particle deposition as well as modifying their retention. For example, breathing mode, i.e., tidal volume and rate, and particle size could be altered thereby changing the site of particle deposition. Selecting appropriate particle characteristics such as size and solubility in water, lipids, or acids can be used to adjust retention kinetics in the respiratory tract once the compound is deposited. Likewise, the use of particles as carriers for compounds to be delivered, such as encapsulation of solutes into liposomes or administration of compounds in hollow polymer microparticles (Worthy, 1988), might be considered. The liposomes or microparticles are then taken up by alveolar macrophages and the encapsulated substance is released after phagocytosis. A new technique for the delivery of compounds via the aerosol bolus technique may also be used, as recently described by Bennett (1991): A bolus of an aerosol is inhaled during a certain phase of a breathing cycle and the inhaled aerosol bolus will then preferentially be deposited in a predetermined area of the respiratory tract. Future development in the delivery of drugs to the respiratory tract both for local as well as systemic treatment will certainly advance our capability to therapeutically intervene in diseased states; successful application of such new techniques will have to consider basic clearance and retention mechanisms of compounds inhaled as aerosols.

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Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 12/18/2018 3:46:25 PM
To: Harvey Clewell [HClewell@ramboll.com]
CC: Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Jerry Campbell [JCampbell@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; cvanlandingham@ramboll.com [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usereda39e51]; Sonja Sax [SSax@ramboll.com]; HIMMELSTEIN, MATTHEW W [Matthew.W.Himmelstein@dupont.com]; Schlosser, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=121cf759d94e4f08afde0ceb646e711b-Schlosser, Paul]
Subject: RE: Chloroprene In Vitro model

Harvey,

Sorry for taking so long.

The vial volume is wrong. Below (scroll down, black text below the separator line) is from the IISRP report. Gas-liquid transfer will be a function of the surface area in the vial, but also the amount of mixing/turbulence in the aqueous phase. The latter will be a nonlinear function of the vial diameter…. Primates have turbulent airflow in the nasal vestibule, rodents do not, because the size of a primate nose is large enough to allow it. More turbulence = faster transfer to the surface.

While they might not be able to obtain the exact vials that Matt did, something close (11-12 mL total volume, similar height/diameter) should be available. They should then measure the total volume as described below, so you have an exact value for the headspace volume. This parameter should then be set in each model script according to the volume of the experiment, but round to reasonable accuracy (+/- 0.1 mL).

Mixing **(Matt?) When I ran incubations, manual sampling, the water bath was set to give gentle shaking, to keep the microsomes suspended/mixed. Matt did this also? Do we know rpm? I don't see it stated in the IISRP report. This will impact equilibration.

Salts, etc: I don't believe these will have much effect on partitioning, but why not replicate the buffering solution used in the original experiments? "0.1 M phosphate buffer (pH 7.4), MgCl2 (15 mM), EDTA (0.1 mM), glucose-6-phosphate (10 mM), and glucose-6-phosphate dehydrogenase (2 U/mL)"

If the smaller volume of the "incubation" vial leads to sampling issues, getting enough CP to measure accurately, the concentration of CP added could be increased: add 0.5 cc of 800 ppm CP. If that's going into ~ 10 mL of headspace, the initial concentration should be ~ 40 ppm, so the sample size can be reduced ¼ and still have the same detection sensitivity. Unless we're approaching the solubility limit of CP in water, the mass transfer should be proportional to CP concentration.